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Rethinking the Pertussis vaccine: Formulation of lipid nanoparticles with vaccine adjuvants to achieve enhanced immunity.

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AN ONLINE EVENT
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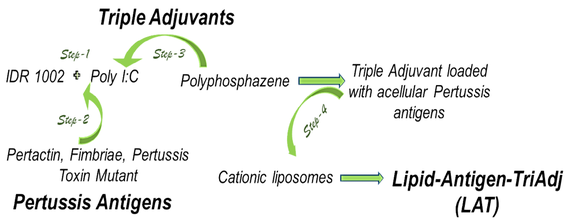
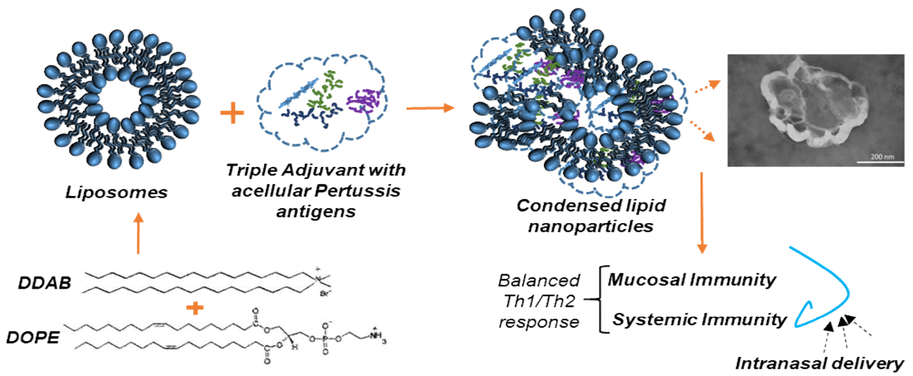
Purpose

- Pertussis or whooping cough is a lungs and pulmonary airways infection which is globally endemic.
- Recent outbreaks of Pertussis have revealed that effectiveness of the current acellular Pertussis vaccine is slowly diminishing, leading to the need for making the existing vaccines better[1].
- A triple adjuvant consisting of a TLR agonist (Poly (I:C)), an immunostimulant host defense peptide (IDR 1002) loaded in a carrier system (polyphosphazene) has achieved improved immune responses[2,3].
- Formulation of this triple adjuvant into cationic lipid nanoparticles for intranasal delivery of pertussis antigens can provide efficient mucosal adhesion and induce a mucosal and systemic immune response[4].

Methods

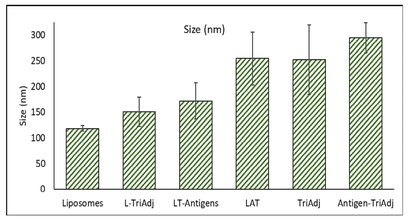


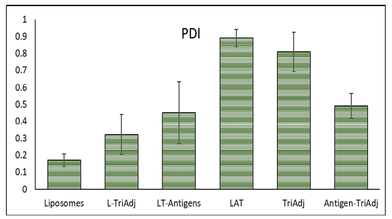
- Blank cationic liposomes composed of DDAB: DOPE (1:1 mol:mol) were prepared using extrusion method.
- Two components of the triple adjuvant namely IDR 1002 peptide and Poly (I:C) were mixed first and kept at room temperature for 30 minutes.
- Acellular Pertussis antigens-Pertussis Toxin Mutant (PTM), Pertactin (PRN) and Fimbriae 2/3 (Fim 2/3) were premixed and added to the IDR-Poly(I:C) mixture and kept at room temperature for 30 mins.
- Polyphosphazene was then added and kept at room temperature for 30 mins to envelop the adjuvants loaded antigens to form the Antigen-TriAdj.
- Finally liposomes and Antigen-TriAdj were mixed and kept on ice for 30 mins to form the Lipid-Antigen-Triadj (LAT).
- Antigens were also loaded in L-Triadj by a second physical mixing method of adding the antigens after preparing the L-TriAdj and named as LT-Antigens.

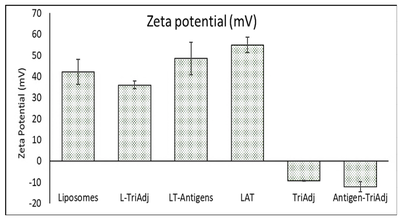


Results

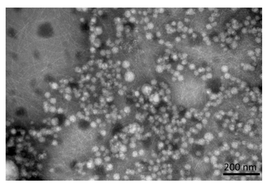
- The negatively charged TriAdj and Antigen-TriAdj interact with positively charged liposomes to form L-TriAdj and LAT with charge +40-50mV
- Blank liposomes having a size of about 100nm when formed into L-TriAdj increases the size to 150nm.
- Physical mixing of L-TriAdj with antigens showed size of 170nm which is not a significant increase as compared to plain L-TriAdj indicating the antigens did not interact with the L-TriAdj.
- Addition of Pertussis antigens at the TriAdj assembling stage allowed for their maximum incorporation into the formulation and forming the LAT which increased the size to 255nm without affecting the zeta potential.

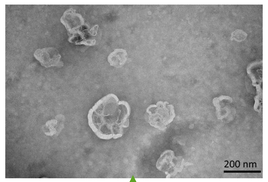






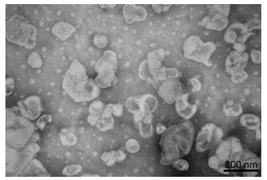
TEM of acellular Pertussis antigens indicate that Pertactin and Pertussis Toxin Mutant are globular proteins whereas fimbriae are filamentous proteins.

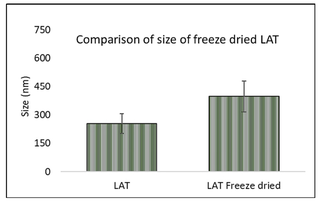




TEM of LAT containing all three pertussis antigens showed the lipidic system encompassing all the antigens along with the Triadj into a single system.

TEM of physically mixed pertussis antigens to preformed L-TriAdj indicated that antigens do not fully associate with the L-TriAdj





Freeze drying is essential to achieve long term stability. Freeze dried LAT formulations had a larger size upon reconstitution as compared to non freeze dried LAT.

Conclusion

- Intranasal vaccines can have far-reaching impacts on the society since majority of people especially children prefer nasal sprays over taking injections.
- Preliminary formulation of mixing Pertussis antigens with the L-TriAdj indicated that the antigens do not successfully attach to the L-TriAdj but co-exist in solution with it.
- The modified formulation approach to include antigens at the TriAdj preparation stage and later interacted with liposomes to form Lipid-Antigens-TriAdj (LAT) system was successful.
- The LAT system allowed complete integration of the antigens into the formulation demonstrated by the size and TEM of LAT nanoparticles.
- These lipid based triple adjuvant nanoparticles can be utilized for intranasal vaccine delivery and have broad applications for various therapeutic and vaccine formulations.

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